American Journal of Hematology 64:71–72 (2000)

Folate Sufficient Subjects Do Not Accumulate Additional Folates During Supplementation

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In a double-blinded placebo-controlled trial of folic acid supplementation in 82 alcoholic subjects, it was found that whole blood folate levels, determined by a mass spectrometric method, do not increase in subjects whose baseline folate levels are above the third quartile (folate sufficiency). Since a state of folate sufficiency can now be identified, a recommended daily allowance (RDA) for folate can be determined using objective means. Am. J. Hematol. 64:71–72, 2000. © 2000 Wiley-Liss, Inc.

Key words: folate assays; insufficiency; deficiency

Subclinical folate deficiency is felt to contribute to the pathogenesis of several conditions including birth defects, cardiovascular disease, cancer, and mental illness [1]. Recent legislation in several countries has led to fortification of common foods with folic acid primarily to prevent birth defects. Concern [2] has been expressed on the dangers of long term folate supplementation and it is not known whether supraphysiologic folate intake results in supraphysiologic tissue levels.

It is, therefore, important to identify subjects at risk of complications of *subclinical* folate deficiency or conversely, subjects who are folate-sufficient. Use of commercially available folate [3] or homocysteine [4,5] assays have been unsuccessful in identifying *sub*clinical deficiency due to poor comparability, assay accuracy [3], or marked variation from subject to subject [4]. We hypothesize that, conversely, folate sufficient subjects should be able to be identified. These subjects should not show incremental increase in tissue (whole blood) folate during supplementation. We tested this hypothesis by measuring changes in whole blood folate (WBF) during supplementation using a verified, accurate mass spectrometric molar folate analysis [6].

METHODS

Studies were approved by the IRB and informed consent obtained from all subjects. Initial folate status was determined in 106 subjects at risk of folate deficiency (alcoholic subjects admitted to hospital for rehabilitation on a voluntary basis). Afterward, 82 of these subjects (11 women and 71 men, mean age of 37 years) continued in a double-blind study of placebo-controlled folic acid (1 mg tablets daily) administration. Molar WBF levels were determined according to published methods using a previously verified mass spectrometric folate assay [6].

RESULTS

The average molar WBF value at baseline was 3.12 nmol/gm Hb (95% CI 2.83–3.41, reference range 1.69–6.69). The follow-up folate values were determined after 18.5 days (95% C.I. 16.8–20.2) of therapy. The subjects receiving folate increased their WBF levels to 3.63 nmol/gm Hb (95% C.I. 3.30–3.96, $P = 0.01$, two-tailed Student's *t*-test), while the changes in WBF in subjects receiving placebo was not significant ($P = 0.59$).

We then determined the change in WBF compared to the initial WBF value. The baseline folate levels and absolute increases for the entire group on folate supplementation were inversely related (Pearson $r = 0.35$, $P \leq 0.01$) as expected. Figure 1 shows the absolute increases in WBF in all the subjects grouped by quartiles using

Contract grant sponser: NIH; Contract grant number: R44 DK50519.

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Received for publication 5 October 1999; Accepted 1 December 1999

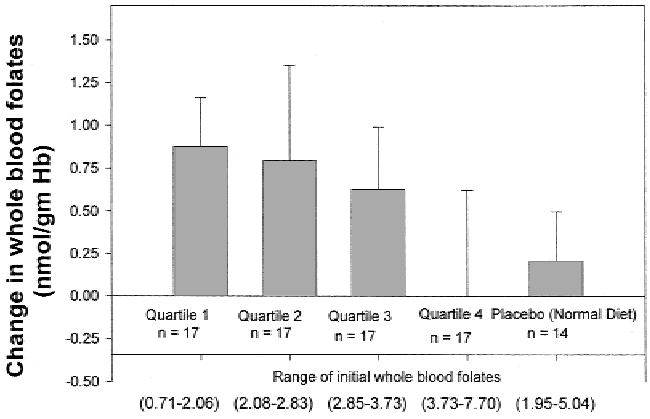

Fig. 1. Absolute increase in molar WBF in 82 subjects on an inpatient rehabilitation ward. Subjects were placed on a normal diet and supplemented with either 1 mg folic acid daily or placebo. Supplemental subjects are grouped in quartiles based on the initial WBF value. The placebo group is the fifth column. Solid bars represent mean absolute change in WBF three weeks after entering the study. The error bars show the standard deviation. Unpaired *t*-testing showed a significant difference between the mean of quartile 1 and quartile 4 ($P = 0.01$), between quartile 2 and quartile 4 ($P = 0.05$), and between quartile 1 and placebo ($P = 0.002$). There was no difference between quartile 4 and placebo ($P = 0.55$) or between quartiles 2 and 3.

baseline WBF values. The increments in WBF were most marked for subjects with lower baseline values. There was no increase in folates in the supplemented subjects with initial folate values in the uppermost quartile. The difference in folate increments was significant between the lowest quartile and the highest quartile ($P = 0.01$, two-tailed Student's *t*-test). The folate increments were also significantly different between the placebo group and the lowest quartile of the treatment group ($P = 0.01$), whereas there was no difference in folate increments between placebo and subjects in the upper quartile ($P = 0.65$).

DISCUSSION

Our data show that subjects with folate values in the uppermost quartile do not assimilate additional folates during supplementation and are therefore *folate sufficient*. In the acute setting, human and animal experiments have shown that folate absorption in the intestine and folate reabsorption in the renal tubules are saturable [7]. Since regulation of the metabolism of vitamins occurs at different levels such as during digestion, absorption, storage or excretion, tissue levels (RBC or whole blood) as opposed to serum or plasma levels may give a better indication of tissue sufficiency [8,9]. The current study indicates that total tissue folate accumulation may be saturable. We propose that saturation of accumulation mechanisms occurs when *folate sufficiency* is reached. Our findings also indicate that dietary supplementation of folate may not be deleterious since inordinate increases in tissue folates do not occur during short term supplementation with 1 mg/day folic acid. Since we now appear to be able to identify a subset of the population who are folate sufficient, more definitive analyses of folate status can be carried out. This information can be added to data collected by investigators using other methods to determine optimal folate intake [10]. Additional studies with longer intervals and varying doses of folic acid may enable us to determine the optimal dietary intake and consequently the RDA for folate.

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